Effect of Triterpenoids and Limonoids Isolated from *Cabralea canjerana* and *Carapa guianensis* (Meliaceae) against *Spodoptera frugiperda* (J. E. Smith)

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The activities of two triterpenoids, ocotillone and cabraleadiol, and four limonoids, methyl angolensate, 3-*β*-deacetylfissinolide, 7-deacetoxy-7-oxogedunin, and *β*-photogedunin, isolated from arillus of *Carapa guianensis* and fruits and seeds of *Cabralea canjerana* (Meliaceae), were evaluated against the fall armyworm *Spodoptera frugiperda.* Gedunin was used as a positive control. 7-Deacetoxy-7-oxogedunin and *β*-photogedunin reduced the pupal weight as occurred with gedunin. Cabraleadiol, 3-*β*-deacetylfissinolide, and 7-deacetoxy-7-oxogedunin prolonged the larval phase similar to the control (gedunin) of approximately 1.2 days at 50.0 mg kg^{-1}. The highest insecticidal activity was obtained for *β*-photogedunin.

Key words: Spodoptera frugiperda, Limonoids, Triterpenoids

Introduction

In the last decades considerable efforts have been devoted to the discovery of new sources of botanical insecticides and antifeedants. Among the plant families studied, Meliaceae and Rutaceae are perhaps the most promising (Schoonhoven, 1982), at least partly due to the presence of limonoids and triterpenoids characteristic of the Rutales order. The biological activity of limonoids from Rutales has been reviewed (Champagne *et al*., 1992). Some neem-based botanical insecticides have already been developed and marketed (Schmutterer, 1990, 1995, 2005).

Carapa guianensis Aublet, also called andiroba, andiroba-saruva, iandirova, carapá, and nandiroba (Lorenzi and Matos, 2002), is a tall Meliaceae tree growing wild throughout South America, West India, and South Africa. In Brazil, it can be found prevalently in flooded areas of the Amazon rainforest (Ambrozin *et al.*, 2006). From the nuts of this plant an oil, called andiroba oil, is extracted, which has a long history of traditional use in South America, as analgesic, anti-inflammatory, insecticide, antibacterial, antiparasitic, and as an anticancer remedy (Bickii *et al.*, 2000; Gilbert *et al.*, 1999; MacKinnon *et al.*, 1997; Moura *et al.*, 2002). Andiroba oil is rich in fatty acids such as oleic, palmitic, stearic, and linoleic acids, together with 2–5% of unsaponifiable material. In addition, from several parts of *Carapa guianensis* limonoids, triterpenes, steroids, coumarins, flavonoids, and diglycerides have been isolated (Lavie *et al.*, 1973; Marcelle and Moto, 1975; Ollis *et al.*, 1964, 1970; Qi *et al.*, 2003, 2004).

Cabralea canjerana (Vell.) Mart. (Meliaceae) ("canjarana") is a native tree of economic importance in Brazil. It is a dioecious tree with widespread distribution in the neotropics, extending from Costa Rica to southern Brazil and northern Argentina. According to Pennington *et al.* (1981), it inhabits mainly nonflooded evergreen lowland or lower hill rain forests.

In the present paper, triterpenoids and limonoids isolated from *C. canjerana* and *C. guianensis* were examined for their effects on the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). *S. frugiperda* is a major pest of many crops in the Americas and one of the most important pests of tropical maize, causing up to 34% reduction in the overall productivity of this crop in Brazil (Cruz *et al.*, 1996).

Material and Methods

Plant material

Cabralea canjerana was collected at the Universidade Federal de Viçosa, Viçosa, MG, Brazil, and identified by T. D. Pennington from the Royal Botanic Garden, Kew, UK. A voucher (1842; 1983) is deposited in the herbarium of Departamento de Engenharia Florestal Dendrologia, Universidade Federal de Viçosa, Viçosa, MG, Brazil.

The arillus of the *Carapa guianensis* seeds were supplied by Cognis Brasil Ltda., Av. Nações Unidas 10989, 4° andar, São Paulo, SP, Brazil.

Compounds isolation

Fruits (460 g) and seeds (200 g) from *C. canjerana* were extracted with ethanol, at room temperature for three times. The ethanolic extract (88.3 g) of the fruits was submitted to liquid-liquid partition with 120 ml methanol/water (3:1, v/v) solution. This solution was partitioned with different solvents to give fractions in *n*-hexane (fr-Hex, 47.4 g), dichloromethane (fr-CH_2Cl_2, 5.4 g), ethyl acetate (fr-EtOAc, 4.4 g), and methanol (fr-MeOH, 11.9 g). The fraction fr-Hex (2.8 g) was submitted to a silica gel column (230–400 mesh, 4.0 x 16.0 cm i.d., 45 fractions of 50 ml) using *n*-hexane/dichloromethane/ethyl acetate/methanol of increasing polarity as mobile phase, yielding nine fractions (fr-Hex.1 to fr-Hex.10) after TLC analysis. The fraction fr-Hex.2 (102 mg) was rechromatographed by column chromatography using Sephadex LH-20 as stationary phase (column 3.2 x 64.0 cm i.d., 26 fractions of 10 ml each) with isocratic elution (methanol/dichloromethane, 1:1, v/v), yielding three fractions (fr-Hex.2.1 to fr-Hex.2.3) after comparison by TLC analysis. The fraction fr-Hex.2.2 (88.6 mg) was rechromatographed on a silica gel column (230–400 mesh, 3.0 x 16.0 cm i.d., 10 fractions of 5 ml each) eluted with *n*-hexane/acetone (9:1, v/v) to afford ocotillone (**1**, 16.3 mg) and cabraleadiol (**2**, 24.4 mg).

The ethanolic extract (14.3 g) of the seeds of *C. canjerana* was chromatographed on microcrystalline cellulose-D and silica gel (column 5.0 x 22.0 cm i.d., fractions of 250 ml each) using *n*-hexane (250 ml) followed by dichloromethane (1 l), acetone (1 l), and methanol (1 l) as mobile phases yielding 13 fractions (EES-1 to EES-13). The fraction EES-2 (1.72 g) was chromatographed on a silica gel column (230 – 400 mesh, 4.0 x 16.0 cm i.d., 69 fractions of 10 ml each) using *n*-hexane/acetone/methanol of increasing polarity as mobile phase to yield eleven fractions (EES-2.1 to EES-2.11) which were joined based on TLC. The fraction EES-2.6 (293.4 mg) was rechromatographed by column chromatography using a Sephadex LH-20 column (3.2 x 64.0 cm i.d., fractions of 10 ml each) with isocratic elution (methanol), yielding fifteen fractions (EES-2.6.1 to EES-2.6.15). The fraction EES-2.6.7 afforded methyl angolensate (**3**, 48.2 mg). The fraction EES-2.7 (230.6 mg) was rechromatographed on a silica gel column (230–400 mesh, 4.0 x 16.0 cm i.d., 35 fractions of 10 ml each), using *n*-hexane/ethyl acetate (8:2, v/v) as mobile isocratic phase, yielding seventeen fractions (EES-2.7.1 to EES-2.7.17). The fraction EES-2.7.9 afforded 3-*β*-deacetylfissinolide (**4**, 63.7 mg).

The arillus ethanolic extract (10.0 g) of *C. guianensis* was submitted to vacuum chromatography using silica gel as stationary phase (70–230 mesh, column 30 x 5 cm i.d.) and *n*-hexane followed by CH_2Cl_2, EtOAc, and MeOH (1 l each) as mobile phases, yielding four fractions (H, D, Ac, Me). The fraction Ac (3.8 g) was submitted to column chromatography over silica gel (230–400 mesh, 30 x 4 cm i.d., *n*-hexane and acetone gradient as eluent, starting with pure *n*-hexane and finishing with pure acetone, 32 fractions of 200 ml each) to give five fractions (Ac1 to Ac5). The fraction Ac3 (3.0 g) was rechromatographed on a silica gel column (230–400 mesh, 10 x 3 cm i.d.) eluted with solvents of increasing polarity (CH_2Cl_2 to MeOH, 28 fractions of 50 ml each) to yield ten fractions (Ac3.1 to Ac3.10). The limonoids 7-deacetoxy-7-oxogedunin (**5**, 24.4 mg) and *β*-photogedunin (**6**, 14.4 mg) were isolated from fraction Ac3.2 by recycling HPLC (Asahipack polymeric column; eluent, methanol/dichloromethane, 7:3, v/v; flow, 4 ml min^{-1}).

Biological activity

Larvae of *Spodoptera frugiperda* (J. E. Smith) were obtained from the Insect Bioassay Laboratory of Universidade Federal de São Carlos, São Carlos, SP, Brazil, and reared on semi-artificial diets (Kasten *et al*., 1978; Parra, 1986). They were maintained in an incubation chamber with a photoperiod of 12 h light/12 h dark, (70 ± 5)% relative humidity, and (25 ± 1) °C.

For each treatment and control, 30 neonate larvae of *S. frugiperda* were used. A solution of compound was added to ascorbic acid (1.56 g; an

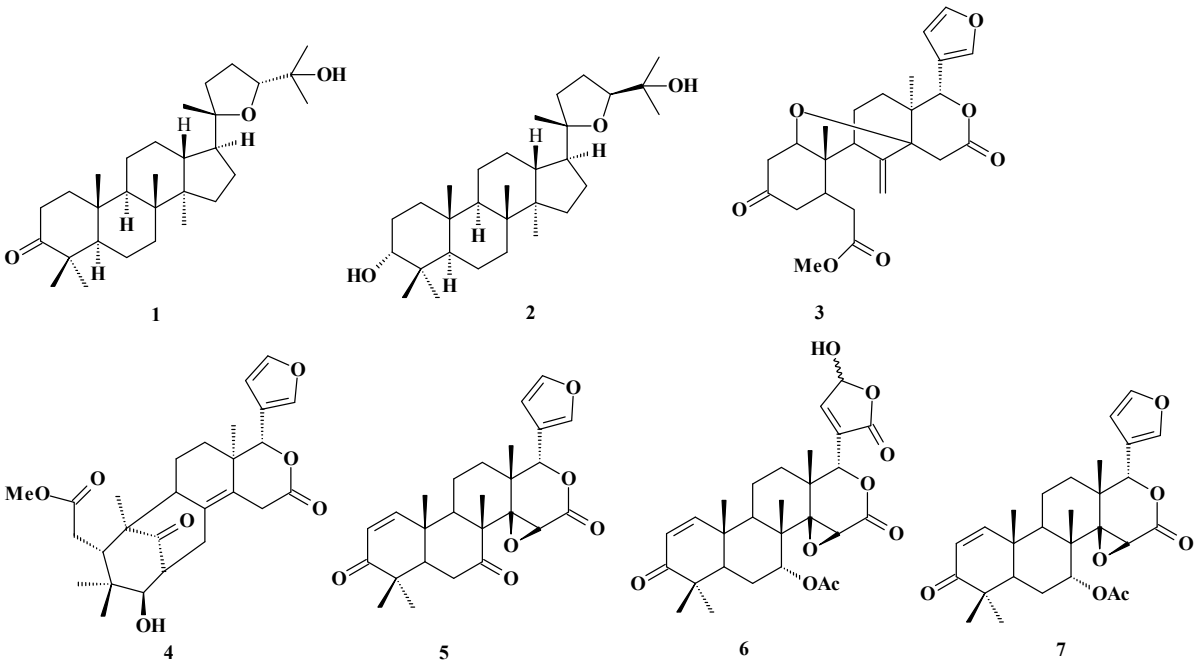

Fig. 1. The triterpenoids ocotillone (**1**) and cabraleadiol (**2**), and the limonoids methyl angolensate (**3**), 3-*β*-deacetylfissinolide (**4**), 7-deacetoxy-7-oxogedunin (**5**), and *β*-photogedunin (**6**) isolated from *Carapa guianensis* and *Cabralea canjerana*, and of gedunin (**7**).

ingredient of the diet). After evaporation of the solvent, the mixture was incorporated into the semi-artificial diet in which bean and wheat germ are the basic ingredients (Kasten *et al.*, 1978) at final contents of 1.0, 10.0, 50.0, and 100.0 mg kg^{-1} for 7-deacetoxy-7-oxogedunin (**5**), and 1.0, 10.0, and 50.0 mg kg^{-1} for ocotillone (**1**), cabraleadiol (**2**), methyl angolensate (**3**), 3-*β*-deacetylfissinolide (**4**), and *β*-photogedunin (**6**). The diet for the control was prepared similarly but without the triterpenoids or limonoids. Gedunin (**7**) was used as positive control. The diets were placed in previously sterilized glass tubes (8.5 cm × 2.5 cm), into which larvae of *S. frugiperda* were introduced individually. The pupae were weighed 1 d after pupation and were transferred to plastics cups, where they stayed until the emergence of adults. Daily observations were made and the following parameters were evaluated: (1) duration of larval and pupal phases, (2) weight of pupae, and (3) percentage of dead insects (mortality) at the end of each phase.

Statistics

Data were submitted to an analysis of variance ANOVA (Zar, 1974), and the averages were compared applying the Tukey test ($P \leq 0.05$). Each tube containing one insect, independent of the developed phase, was considered as one replicate, therefore, the number of replicates was different for each treatment. For evaluation of the mortality of the larvae and pupae, the experimental unit was constituted by the mean of five tubes containing one larva each, with six replications by treatment.

Results and Discussion

The triterpenoids **1** and **2** and limonoids **3** and **4** were isolated from the fruits and seeds of *C. canjerana* and the limonoids **5** and **6** from the arillus of *C. guianensis* (Fig. 1). Their structures were confirmed by ^{1}H and ^{13}C NMR spectroscopy and comparison with data from the literature (Braga *et al.*, 2006; Ambrozin *et al.*, 2006).

Compounds **1**–**6** were incorporated in the semi-artificial diet. **2**, **4**, and **5** affected larval development (Table I), and only **6** affected pupal development at concentrations of 10 and 50 mg [(8.5 ± 1.64) days and (8.9 ± 1.25) days, respectively, control (10.1 ± 1.45) days]. Larvae fed with

Table I. Mean duration of larval stage, weight of pupae, and larvae mortality in *Spodoptera frugiperda* treated with triterpenoids and limonoids administered in artificial diet (n = 30).

Compound or control	Content [mg kg^{-1}]	Larval phase duration [d] (± SD)[a]	Pupal weight [mg][a]	Larvae mortality (%)[a]
1	1	17.6 (± 1.25) a	259.7 a	10.0 b
	10	18.0 (± 1.72) a	255.7 a	16.7 b
	50	18.1 (± 1.43) a	253.9 a	40.0 a
Control		17.4 (± 1.39) a	260.8 a	6.7 b
2	1	18.2 (± 1.61) ab	271.7 a	13.3 a
	10	18.7 (± 2.33) a	271.6 a	20.0 a
	50	19.1 (± 1.67) a	273.3 a	26.7 a
Control		17.4 (± 1.39) b	260.8 a	6.7 a
3	1	18.2 (± 2.39) a	262.6 a	13.3 ab
	10	18.5 (± 2.17) a	263.9 a	33.3 ab
	50	19.3 (± 1.99) a	266.6 a	40.0 a
Control		17.4 (± 1.39) a	260.8 a	6.7 b
4	1	17.1 (± 1.97) ab	264.5 a	10.0 a
	10	18.2 (± 2.06) a	262.7 a	10.0 a
	50	18.5 (± 2.34) a	269.5 a	20.0 a
Control		17.4 (± 1.39) b	260.8 a	6.7 a
5	1	16.3 (± 3.03) ab	247.8 ab	23.3 ab
	10	16.3 (± 1.97) ab	245.3 ab	20.0 ab
	50	16.3 (± 1.18) a	244.7 ab	33.3 ab
	100	17.5 (± 2.48) a	236.7 b	40.0 a
Control		14.9 (± 0.80) b	262.5 a	10.0 b
6	1	24.2 (± 4.25) a	242.7 b	20.0 ab
	10	25.7 (± 3.71) a	241.6 b	33.3 ab
	50	23.3 (± 4.41) a	241.2 b	53.3 a
Control		22.7 (± 3.00) a	272.7 a	10.0 b
7	1	16.6 (± 3.1) a	246.2 a	16.7 b
	10	16.3 (± 3.1) a	245.6 a	40.0 b
	50	16.2 (± 2.0) a	235.3 b	63.3 ab
	100	16.0 (± 0.6) a	238.3 b	80.0 a
Control		14.9 (± 0.8) a	262.5 a	10.0 b

[a] Means followed by the same letters within columns indicate no significant difference ($P \leq 0.05$) in the Tukey test.

semi-artificial diet treated with **5** and **6** showed significant reduction in the pupal weight (17.8 and 31.5 mg at 50 mg kg^{-1}, respectively), comparable to the effect of gedunin (**7**, 27.2 mg, Table I). Larvae fed with semi-artificial diet treated with **2**, **4**, and **5** at 50.0 mg kg^{-1} prolonged the larval time for approximately 1.2 days similar to gedunin, suggesting that these compounds act as larval growth inhibitors (Table I).

Compounds **2** and **4** prolonged the larval phase, although no alteration in pupal weight was observed. Tanzubil and McCaffery (1990) observed that larvae treated with low doses of azadirachtin produced pupae with a weight comparable to that of the controls, suggesting that they did not experience any severe feeding inhibition.

Compound **5** also effected prolongation of the larval phase followed by reduction of the pupal weight. The larvae treated with **5** showed prolongation of the larval phase by 1.4 days (at 50.0 mg kg^{-1}) and 2.6 days (at 100.0 mg kg^{-1}) when compared with the control (14.9 days, Table I). The reduction of the pupal weight was by 17.8 and 25.8 mg at 50.0 and 100.0 mg kg^{-1}, respectively, when compared with the control (262.5 mg, Table I).

Larvae treated with **6** at 1.0, 10.0, and 50.0 mg kg^{-1} presented reduction of the pupal weight by 30.0, 31.1, and 31.5 mg, respectively, when compared with the control (272.7 mg, Table I). This compound reduced the duration of the pupal phase by 1.2 days and that of the larval phase was increased by 0.6 days, while gedunin affected

only the larval phase by 1.3 days at 50 mg kg^{-1} (Table I).

Ocotillone (**1**) isolated from the leaves of *Dysoxylum malabaricum* (Govindachari *et al*., 1994) was tested against *Spodoptera litura* and showed excellent PFI (percentage feeding index) values comparable to those of azadiradione, epoxyazadiradione, and limonin (Govindachari *et al*., 1995).

Methyl angolensate (**3**) exhibited insecticidal activity against *S. litura* in no-choice tests (Abdelgaleil and Nakatani, 2003; Suresh *et al.*, 2002; Powel *et al*., 1991), and this result agrees with that observed in the present work. Isolated from diethyl ether and acetone extracts of the stem bark of *Khaya senegalensis* (Meliaceae) **3** showed antifeedant activity by the conventional leaf disc method on the third-instar larvae of *Spodoptera littoralis* (Boisd., Lepidoptera: Noctuidae) at 500 μg ml^{-1} (Abdelgaleil and Nakatani, 2003; Suresh *et al.*, 2002) in a study examining structure-insect antifeedant relationships of plants belonging to the Rutales order. In a dual-choice antifeedant bioassay against *Spodoptera litura* L. (Lepidoptera: Noctuidae), methyl angolensate (**3**) showed a PFI of 65.3, near to that of cedrelone (51.5). Two limonoids (sandoricin and 6-hydroxysandoricin) isolated from seed extracts of *Sandoricum koetjape* Merr. (Meliaceae), which possess chemical structures similar to that of methyl angolensate (**3**), showed significant antifeedant activity against the fall armyworm (*S. frugiperda*), incorporated in diet at 25 ppm, and similar activity against the European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), at 200 ppm. Toxicity results for both insects indicated that continued feeding on diets containing sandoricin or 6-hydroxysandoricin at the 200 ppm level would result in nearly 100% mortality prior to pupation (Powell *et al*., 1991).

β-Photogedunin (**6**) presented the highest insecticidal activity against *S. frugiperda* causing mortality of 53.3% in the larval stage and 20.0% in the pupal phase at 50 ppm. These results agree with those of Céspedes *et al.* (2000), who tested an epimeric mixture of photogedunin and their corresponding acetates and observed that these compounds caused significant mortality of *S. frugiperda* larvae (45% to 100% at 10.0 to 52.0 ppm) after 7 days, as well as growth reduction, and photogedunin acetates showed the highest insecticidal activity at 10.0 ppm with 17% survival.

Compounds **1**, **3**, **5**, and **6** showed moderate insecticidal activity at 50.0 mg kg^{-1} with 40.0, 40.0, 33.3, and 53.3% mortality, respectively, for the larval phase. Although the compounds tested here were less active than the positive the control gedunin (**7**, 63.3%), the use of these compounds, in particular **6**, is suggested for the control of *S. frugiperda*. Comparison of the activities of **6** and **7** suggests that oxidation of the furan ring led to a decrease in the insecticidal activity.

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